Remarks

Claims 26-50 are pending. Claim 26 has been amended.

Support for the addition of the phrase "the following steps in the order recited" to claim 26 is found at least in paragraph [0008], bridging pages 3 and 4 of the specification. Specifically, this paragraph highlights the importance of performing the complete assay in the shell prior to imaging. Support for the phrase "directly injecting into" is found at least in paragraph [0031] on page 11, wherein cannulation of a vessel is described as a means for administration. Finally, the addition of the phrase "digitally quantifying the plurality of pixels from the three-dimensional image" is found at least in paragraph [0039] on page 13, where the conversion of analog light into a digital signal of pixels is described, and in paragraph [0055] on page 17, where the summing of the pixels to determine the FVD value is disclosed. Thus, no new matter is added by these amendments.

Applicants gratefully acknowledge the rejoinder of Group I (claims 26-40) with elected Group II (claims 41-50).

Applicants also gratefully acknowledge the withdrawal of the previous enablement rejection of claims 26-50 under 35 U.S.C. § 112, first paragraph.

Applicants also gratefully acknowledge the withdrawal of the previous written description rejection of claims 26-27, 29-41, and 43-50 under 35 U.S.C. § 112, first paragraph.

Applicants also gratefully acknowledge the withdrawal of the previous rejection of claims 37-40 and 41-50 under 35 U.S.C. § 112, second paragraph.

Applicants also gratefully acknowledge the withdrawal of the previous rejection of claims 26-40 under 35 U.S.C. § 103(a) as being unpatentable over Brooks et al., Kurz et al, Frasca et al., and Kinnman et al.

Rejection Under 35 U.S.C. § 103

A. The rejection of claims 26-34, 36-40 is maintained under 35 U.S.C. § 103(a) as being unpatentable over Brooks et al (Science. 1994. 264:570-71), Roberts et al. (Cancer Res. 1992.

52(4):924-30), and Kimel et al. (SPIE. 1996. 2628:69-66). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

According to the Office Action, Brooks et al. describes the use of a CAM assay for measuring angiogenic and anti-angiogenic activity, but does not teach administering florescent-labeled particles before removing the test region of interest and capturing the 3D image of the test region to quantitate angiogenesis in the test area. The Office Action then posits that the teaching of Roberts et al. makes up for this deficiency. Specifically, Roberts et al. describe a method of injecting chick embryos with photo sensitizers (fluorescent), which allegedly have a selective affinity for proliferating neovasculature. The Office action then attempts to link this use in tumors to the CAM by pointing to the evaluation of porphyrin bio distribution in the CAM by Kimel et al. The Office Action therefore posits that "it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to modify the method to measure angiogenic or anti-angiogenic activity in a CAM assay of Brooks by measuring the fluorescent vascular density taught by Robert/ Kimel with a reasonable expectation of success." The Office Action then suggests that the artisan would have been motivated to optimize the treatment routes. However, this is not a true characterization of the teaching of Roberts et al. and Kimel et al. as neither of these references evaluated "fluorescent vascular density" as taught or claimed in the instant application. Specifically, none of the cited references attempted or suggested digitally quantifying vascular density using a three-dimensional image of the vasculature. On this basis alone, there is insufficient basis to support prima facie obviousness.

However, in order to facilitate prosecution, claim 26, step (e) has been amended to recite "directly injecting into a vessel located in the CAM a fluorescent labeled particle, **such that the fluorescent labeled particle travels through each vessel contained in the test region of interest**." In contrast, the value of having the fluorescent labeled particle travel through each vessel contained in the test region of interest is not taught or suggested by Brooks et al. (Science). This is the case because Brooks et al. administered their fluorescence labeled antibodies after the CAM was removed from the egg and fixed. Brooks et al. state that the "CAMs were snap frozen, and ... sections were fixed with acetone and stained by

immunofluorescence with [antibody]" (Legend of Fig. 2 (A)). Since the CAM with the test region of interest is fixed prior to addition of the fluorescent-labeled particle, it is unlikely, if not impossible, that the fluorescent-labeled particle traveled through each vessel in the test region of interest.

Moreover, claim 26 is further amended to require that the recited steps be carried out in the shell prior to imaging. It is clear from a reading of Brooks et al. that a step of removing the substrate and test region (f) after administering the fluorescent labeled particle (e) is not disclosed in Brooks et al. The Examiner has noted this. Rather, Brooks et al. teach that the "CAMs were snap frozen, and sections were fixed with acetone and stained by immunofluorescence with antibody" (Legend of Fig. 2 (A)). Not only does this limitation distinguish Brooks et al., it also provides an advantage to the present method. More specifically, paragraph [0008] of the specification teaches the advantage of "administration of a fluorescent-labeled particle to the CAM before the angiogenic or antiangiogenic activity is measured" and "by performing the complete assay in the shell, the test molecule can be delivered systemically, as compared to locally, to allow more accurate results to be obtained."

Roberts et al. is further irrelevant to the present method, because they do not capture an image of the test region at all. Rather they measure the amount of specific photosensizers in the CAM by extracting them from the CAM. Thus, the advantage of providing a better image of the CAM by administration of the fluorescent labeled particle prior to removal of the CAM would not have been recognized by the person reading Roberts et al. Thus, this reference does not provide the teaching or suggestion missing from Brooks et al.

Moreover, claim 26 has been amended to require "digitally quantifying the plurality of pixels from the three-dimensional image to obtain a fluorescent vascular density (FVD) value." In contrast, none of Brooks et al., Roberts et al. or Kimel et al. teach the use of digital methods to quantify effects on vessel growth.

Thus, improved imaging and digital quantification of angiogenic or anti-angiogenic activity is not recognized from a reading of Brooks et al., Roberts et al. or Kimel et al. because none of these references had the goal of the present invention, namely, to provide an improved

method of measuring the angiogenic or anti-angiogenic effect of a test compound by quantifying pixels from a three-dimensional rendering of the blood vessels. Since the combination of these references fails to teach all of the limitations of the present steps, the Office Action has not established a *prima facie* case for obviousness. For this reason alone, the present rejection fails and should be withdrawn.

Moreover, even if, *arguendo*, a *prima facie* case were made, the claimed CAM assay provides significant improvements over the CAM assay that were unexpected. As shown in Example 3, on pages 22-24 of the specification, Applicants' CAM assay is superior to previous detection methods in the art. For example, as demonstrated by the results set forth in Tables 2-4, when prior art CAM assays were evaluated by blinded observers using a detection method of the prior art, none of the three independent, blinded observers could visually appreciate differences between the groups when the methodology of blinded grading was applied. However, as indicated in Example 1, differences were seen when an FVD value was assigned to each group according to the claimed method. Thus, Applicants have provided novel CAM methods of measuring angiogenic and antiangiogenic activity that improved the measurement of these activities to a degree that was unexpected.

As further evidence, Applicants submit herewith a Declaration under 37 C.F.R. § 1.132 by Dr. Frank Cuttita, who is Director of the Angiogenesis Core Facility at the National Cancer Institute. Dr. Cuttitta states in his Declaration that the claimed quantitative CAM technology provides substantially superior results to that of Brooks et al. or any other previously available CAM assay. He further asserts that this contradicted the prevailing wisdom in this field by identifying for the first time the scope of the inaccuracy of the gold standard of the time, the Brooks et al. CAM assay. Dr. Cuttitta attributes the improved results in part to the fact that the claimed method is an objective based quantification of vascular density and not a subjective interpretation of dye "retention," dye "biodistribution," or dye "density" as disclosed in Robert et al. and Rizzo et al., Kimel et al., and Brooks et al., respectively.

Dr. Cuttitta's Declaration further states that prior to the filing of the above referenced application, those working in the angiogenesis field did not have reason to believe that the CAM

assay taught by Brooks et al. was unable to accurately quantitate angiogenesis (increase in vascular density) or that there were any alterations to the known CAM assay that would provide significantly superior results. Dr. Cuttitta states that while the prior art CAM assay was understood to have limitations, there was no general recognition in the field that results based on dye density were an inaccurate indicator of vessel density, or that a different approach would give significantly more accurate results.

Dr. Cuttitta further indicates in his Declaration that the superior results of the claimed method were surprising to scientists in this field when they were first disclosed. While some improvement of accuracy was expected with the use of more quantitative measurements of angiogenesis, Dr. Cuttitta states that the significance of the improvement shown by the claimed method was unexpected. In fact it was the surprising degree of improvement by the present method that first alerted those in the art to the scope of the problem with the prior art CAM assay.

Finally, Dr. Cuttitta states in his Declaration that the claimed assay has been commercially successful based on the recognition of the superior results obtained using the objective and direct quantitation of vessel density. He indicates that the results obtained with the claimed method have been lauded by others in the field in the face of competing methods and that this commercial success is directly related to the superior results of this assay over the methods previously used.

Applicants therefore assert that the Declaration under 37 C.F.R. § 1.132 is sufficient evidence that the claimed methods are not made obvious by the combination of Brooks et al, Roberts et al., and Kimel et al. For at least these reasons, Applicants respectfully request the withdrawal of this rejection.

B. The rejection of claims 26-40 is maintained under 35 U.S.C. § 103(a) as being unpatentable over Brooks et al. and Rizzo et al. (Microvascular Res. 1995. 49:49-63). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The Office Action admits that Brooks et al. does not explicitly teach administering FITC labeled particles to measure angiogenic activity. Thus, the Office Action bases this rejection on the position that Rizzo et al. allegedly taught a method of measuring fluorescent vascular density that can be used by the artisan of ordinary skill to modify the CAM assay taught by Brooks et al.

First, even in combination, Brooks et al. and Rizzo et al. do not teach every limitation of the claimed method. For the reasons stated above, Brooks et al. does not provide either a reasonable expectation of obtaining the present method, nor motivation to do so. Furthermore, the Office Action misinterpreted Rizzo et al. in suggesting that it taught a method of "measuring fluorescent vascular density."

As noted by the Office Action, Rizzo et al. actually taught a method to quantitate vascular permeability associated with tumorigenesis and vascular permeability associated with normal angiogenesis by microinjecting a graded series of FITC-dextrans into a vessel of a CAM. The resulting fluorescence that was detected was therefore not a measure of vascular density, but of vascular permeability. There is nothing in Rizzo et al. that would suggest attempting to quantitate new vessel growth, much less to do so by to creating a three-dimensional rendering of the vascular networks and digitally quantitating new vessel growth. In fact, the FITC-dextrans used by Rizzo et al. would not work in the present method due to their size. Therefore, the modification of Brooks et al. to include FITC-dextrans of Rizzo et al would result in a method that is not the claimed method. For this reason alone, the present rejection fails to make out a prima facie case of obviousness.

Consequently, the Examiner is using impermissible hindsight to suggest that the person having ordinary skill would have made the leap from the use of FITC-dextran, where the dextran was small enough to extravasate from leaky blood vessels, for the purpose of identifying leaky blood vessels to the use of larger dextrans that would not leak during vascular permeability for a

completely different purpose, i.e., quantitating pixels from a three-dimensional rendering of the vasculature to measure new vessel formation. Further, the same artisan would have no reason to believe that doing so would provide better results than simply immunolabeling the blood vessels after the tissue was fixed.

Moreover, Applicants respectfully assert for the reasons stated above and as evidenced by the Declaration under 37 C.F.R. §1.132 that the claimed method provided a significant and unexpected improvement over the CAM assay taught by Brooks et al., and that this deficiency is not cured by Rizzo et al. Applicants assert that the Declaration by Dr. Cuttitta is sufficient evidence that the claimed methods are not made obvious by the combination of Brooks et al and Rizzo et al. For at least these reasons, Applicants respectfully request the withdrawal of this rejection.

C. The rejection of claims 41-50 is maintained under 35 U.S.C. § 103(a) as being unpatentable over Brooks et al. (Methods in Molecular Biology. 129:257-269), Kurz et al. (Dev. Dyn. 1995. 203:174-186), Frasca et al. (Oncogene. 2001. 20:3845-56), and Kinnman et al. (Lab Invest. 2001. 81(12):1709-16). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The Office Action recognizes that while Brooks et al. described the potential of measuring angiogenic and anti-angiogenic activity using CAM assays, they did not teach adding an agent to measure metabolic activity. However, the Office Action bases the rejection on the assertion that the use of proliferation-based assays was routinely used in the art to quantitate angiogenic or anti-angiogenic activity. The Office Action supports this position by pointing to the use of BrdU labeling in CAM assays as taught by Kurz et al. The Office Action then cites Frasca et al. and Kinnman et al. to support the position that metabolic markers such as XTT are interchangeable with BrdU as viable means of detecting cell proliferation. However, this is incorrect.

The person of ordinary skill in the art at the time the application was filed would not have presumed that XTT could substitute for BrdU for detecting proliferation in the CAM. While it is

true that BrdU detection is a proliferation assay, and that Kurz et al. taught the use of this method in CAM assays, there is a significant difference between BrdU-based assays and the metabolic assays as claimed. Whereas BrdU can be used with a cell-specific antibody to discriminate the cell type, XTT is indiscriminate. For example, Frasca et al. used XTT to measure proliferation of human thyroid cancer cells in matrigel, and Kinnman et al. investigated proliferation of hepatic stellate cells. Importantly, both of these reports were for homogenous cell cultures and not heterogeneous tissue. This is in contrast to the multiple cell types other than the endothelial cells making up the blood vessels of the CAM. For CAM assays, therefore, the skilled artisan would have believed it necessary to account for the non-endothelial cells when assaying for angiogenic effects. This is the reason why Kurz et al. analyzed BrdU labeled endothelial cells by computer assisted microscopy, which allowed a visual determination of whether a BrdU-labeled cell was in fact an endothelial cell.

Applicants therefore demonstrated surprising results in showing that metabolic substrates, such as XTT, which do not discriminate between cell types, could be used in the CAM assay to monitor the affect of angiogenic and anti-angiogenic agents on proliferation. Specifically, Applicants demonstrated that the use of an angiogenic stimulus that is endothelial-cell specific can be used in combination with a metabolic assay to evaluate candidate anti-angiogenic agents. There was no evidence in the art prior to the present results that such non-discriminatory method could be used to detect significant effects of angiogenic and anti-angiogenic agents in a CAM. As such, the claimed method is not made obvious by the combination of Brooks et al., Kurz et al., Frasca et al. and Kinnman et al. Applicants therefore respectfully request the withdrawal of this rejection and allowance of claims 41-50.

Pursuant to the above amendments and remarks, reconsideration and allowance of the pending application is believed to be warranted. The Examiner is invited and encouraged to directly contact the undersigned if such contact may enhance the efficient prosecution of this application to issue.

A credit card payment submitted via EFS Web in the amount of $810.00, representing the Request for Continued Examination (RCE) fee for a large entity under 37 C.F.R. § 1.17(e), a RCE, and a Declaration under 37 C.F.R. § 1.132 by Dr. Frank Cuttitta are hereby enclosed. This amount is believed to be correct; however, the Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,
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